

## HEMATOLOGY CONTROL AND SYSTEM FOR MULTI-PARAMETER HEMATOLOGY MEASUREMENTS

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### FIELD OF THE INVENTION

The present invention relates generally to hematology control compositions and systems and, more particularly, to a hematology control composition and system used to measure a plurality of parameters in a sample of blood with a multi-parameter automated hematology instrument.

### BACKGROUND OF THE INVENTION

Hematology controls for various automated instruments that measure, for example, red and white blood cell counts and platelet counts, are known in the art and are described in the following U.S. Pat. Nos.: 3,558,522; 3,873,467; 4,179,398; 4,219,440; 4,299,726; 4,324,687; 4,358,394; and 4,436,821 (incorporated by reference herein). Currently, blood analysis requires the use of one or more of several different instruments and, subsequently, different blood samples and blood sample preparations to analyze the various components of blood. Several hematology instruments, however, now have the capability of measuring various parameters of blood without requiring separate sample preparation for each parameter being tested. Such instruments include the Beckman Coulter STKS or Gen-S Systems, the Abbott Cell-Dyn 4000 Hematology System, Bayer ADVIA 120, and the Sysmex XE2100 System. These improved automated instruments can measure one or more of: 1) reticulocytes, 2) red blood cells, 3) nucleated red blood cells, 4) platelets, 5) reticulated platelets, 6) white blood cells, including lymphocytes, monocytes, neutrophils, eosinophils, basophils, and 7) white blood cells with all phenotypes. It would thus be desirable to provide a hematology control composition that could be used as a control in connection with these instruments.

In the preferred embodiment, a hematology control composition for use with a multi-parameter automated hematology instrument includes a liquid suspension of particulates that has characteristics like whole blood. The control  
5 composition includes one or more blood cell components (i.e. components handled or treated to simulate such a component as found in whole blood), or their analogs, that may or may not be fixed, stabilized, or prepared by other treatment prior to final suspension. In different embodiments, the blood cell components or analog materials may be derived from a source that will exhibit  
10 the size, shape or other characteristics of human, animal, or other whole blood. By way of examples, U.S. Patents Nos. 4,198,206; 4,436,821; 5,008,021; 5,262,327; 5,270,208; 5,432,089; 5,672,474; and 5,731,205, which are each hereby incorporated by reference, each contain examples of these types of blood cell components or analogs. The control has one or more blood  
15 component or analog to resemble corresponding components in whole blood when measured by the multi-parameter automated hematology instrument. When so measured, the control composition would assist in the calibration, operation, and accumulation of quality assurance data for the multi-parameter automated hematology instrument.

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### SUMMARY OF THE INVENTION

A hematology control and system for multi-parameter hematology measurement is provided. The hematology control provides values for the various components of blood that the multi-parameter hematology instrument is capable of measuring. The hematology control composition comprises components for simulating reticulocyte, white blood cell, red blood cell, nucleated red blood cell, platelet or reticulated platelet constituents of whole blood.  
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Methods of making and using the hematology control composition of the present invention are also provided herein.

The system of the present invention includes a hematology instrument, a control, and may further include output or readout devices. In one embodiment the system includes other peripheral devices, such as a device for tracking samples and associating them with particular data, such as a bar-code scanner  
5 system.

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The hematology control composition of the present invention comprises components for simulating one or more of the following constituents of whole  
10 blood: reticulocytes, white blood cells, red blood cells, nucleated red blood cells, platelets, or reticulated platelets. In one embodiment the components are suspended in an isotonic medium. The hematology control composition of the present invention provides values for various constituents of blood that a hematology instrument, such as a multi-parameter hematology instrument, is  
15 capable of measuring. Examples of multi-parameter hematology instruments include those available commercially without limitation, under the designations Beckman Coulter STKS or Gen-S Systems, the Abbott Cell-Dyn 4000 Hematology System, Bayer ADVIA 120 System, the Sysmex XE2100 System, or the like.

20 In one embodiment of the present invention, the reticulocyte component of the control composition comprises reticulocytes (that is, immature anucleate red blood cells containing some ribonucleic acid) or an analog thereof. For the present invention, the reticulocyte component is prepared in any suitable manner. By way of example, see U.S. Patent No. 5,432,089, incorporated by  
25 reference. Among possible embodiments, the reticulocyte component may comprise true mammalian reticulocytes prepared for instance by mammalian (e.g. human) red blood cell encapsulation or by isolation from whole blood.

30 The white blood cell component of the hematology control composition comprises a blood cell (e.g. white blood cell) or analog thereof, selected from the group consisting of white blood cells for various cellular types, white blood cells

for all phenotypes, and mixtures thereof. Among the possible embodiments, the white blood cell component may comprise white blood cells that are fixed with a suitable agent; preferably with an aldehyde; and more preferably glutaraldehyde, with glutaraldehyde and formaldehyde, or with 20% Nuosept 145. U.S. Patent 5 Nos. 5,270,208 and 5,262,327, incorporated herein by reference, provide examples of a suitable white blood cell component. Of course, the skilled artisan will appreciate that the subject invention is not limited to white blood cell components prepared from only white blood cells. Analogs prepared from any of a variety of other sources are possible, including but not limited to red blood cells 10 from birds, reptiles, mammals, etc.

The red blood cell component comprises red blood cells or an analog thereof. Among other possible methods of preparation, the red blood cells may be washed free of all other cellular material, such as by using a magnesium gluconate diluent.

15 When employed, the nucleated red blood cell component of the control composition of the present invention comprises nucleated red blood cells or an analog thereof, such as avian red blood cells, e.g., turkey or chicken red blood cells.

In another embodiment of the present invention, the hematology control 20 composition additionally provides a platelet component, preferably a simulated platelet component. Among other possible types, the platelet component may comprise stabilized human platelets or platelets simulated from goat, bovine or porcine blood cells. In one embodiment, they are prepared from red blood cells. See, U.S. Pat. Nos. 4,160,644 and 4,198,206, incorporated herein by reference, 25 disclose an example of a suitable platelet reference control and methods of preparation. The skilled artisan will appreciate a number of other techniques for preparing simulated platelets.

In another embodiment of the present invention, the control composition comprises a reticulated platelet component. To illustrate, without limitation, goat

red blood cells with encapsulated nucleic acids would constitute one example of a reticulated platelet component useful for the present invention.

The components of the control preferably are suspended in a suitable suspension medium that permits the control to be processed through the  
5 automated instrument. By way of example, among the possible embodiments of the present invention, the isotonic suspension medium may comprise a magnesium gluconate/ethylene diamine tetraacetic acid (EDTA)/phosphate buffer with nucleated red blood cells; the same buffer with the additives HDL, sulfasalazine and alpha tocopherol; or the same buffer with 3% albumin.

10 The present invention further relates to a method of using the hematology control composition as set forth above in the analysis of whole blood. In addition, the present invention relates to a method of making a hematology control composition for use with multi-parameter systems, comprising the step of mixing one or more of a reticulocyte (retic) component, a white blood cell component, a  
15 red blood cell component, a nucleated red blood cell component, a platelet component, and a reticulated platelet component in an isotonic suspension medium.

It will be appreciated that the term "control composition" as used herein means one or more blood components and includes naturally occurring blood  
20 components as well as analogs thereof, which when combined or used alone, sufficiently simulate the relevant characteristics of whole blood for which the instrument tests. The following examples address the preparation of various of the constituent components. The control of the present invention contemplates an admixture of two or more of the components.

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#### SPECIFIC EXAMPLE 1

The following example describes the preparation of a preferred control composition of the present invention.

Stock volumes of the constituent components are prepared in the following approximate concentrations:

RBC:	$6.0 \times 10^6/\text{mm}^3$
WBC:	$150,000/\text{mm}^3$
Platelets:	$10 \times 10^6/\text{mm}^3$
Retics:	50% of $5.5 \times 10^6/\text{mm}^3$ red count
NRBC:	$0.5 \times 10^6/\text{mm}^3$

10 To prepare the final control composition, for example, in a 5 liter volume, stock volumes of the constituent components are combined as follows:

		Approximate <u>Target Count</u>	Approximate <u>Volume Stock</u>
15	RBC:	$4.5 \times 10^6/\text{mm}^3$	3,750 ml
	WBC:	$8.0 \times 10^3/\text{mm}^3$	266 ml
	Plt (platelets)	$225 \times 10^3/\text{mm}^3$	112 ml
	Retic (%)	3%	370 ml
20	NRBC	0.01%	4.5 ml

The combined constituents are brought to a final total volume of 5 liters by adding final diluent including SUPERTRATE, prepared according to U.S. Patent No. 5,262,327, incorporated herein. The skilled artisan will appreciate that there are other means and procedures to prepare this and other embodiments of the present invention.

#### SPECIFIC EXAMPLE 2

The following example describes the preparation of a preferred white blood cell component of the present invention.

In a preferred embodiment of the present invention, the white blood cells for cellular types comprise one or more of lymphocytes, monocytes, neutrophils, eosinophils and basophils. When preparing the white blood cell component for the control composition of the present invention, the cells are obtained by 5 standard separation from whole blood or from portion of previously fractionated whole blood containing the desired cell population. The cells are resuspended, for instance, in a phosphate buffered solution containing polyethylene glycol 20,000 (PEG), EDTA and magnesium gluconate with 2% bovine serum albumin. The osmolarity of this solution is preferably sufficient to swell the white blood 10 cells prior to fixation (e.g. about 215 mosm). The cells may then be stored in this solution, e.g. at about 6°C for 1 hour.

The cells are fixed in a suitable media in order preferably to denature the surface or otherwise accomplish preserving the cell morphology. To illustrate, in one embodiment, in a solution of distilled water containing 5 g/l sorbitol, 7.4% 15 formaldehyde and 0.125% glutaraldehyde. Of course, other suitable fixing agents may be used in suitable amounts. In a highly preferred embodiment, the white blood cells and the fix solution are maintained at a temperature sufficient to provide a proper white blood cell position (e.g. between about 4°C and 12°C). The fixative is added to the cells at a suitable ratio. For example, in one 20 embodiment, a ratio of between 10 ml of cell suspension to 24 ml of fix solution is used. The distilled water in the fix solution swells the white blood cells further, while the fixative stabilizes the cell membrane. The cells are thus left in the fixative for 2 days at room temperature.

After fixation, the cells preferably are washed. In one aspect, they are 25 washed in a phosphate buffered solution. One such solution contains PEG, EDTA, magnesium gluconate and bovine serum albumin. Lipoprotein concentrate is added at 150 mg/dl HDL to store the cells prior to use in order to improve the stability of the scattergram position while the white blood cells are waiting to be added to the other components of the control composition.

White blood cells prepared as described in U.S. Patent No. 5,459,073 (incorporated herein by reference) for flow cytometry may be employed for phenotyping. By mixing the two types of white cells, both requirements can be met, i.e., white blood cells for various cellular types and phenotypes, as the cells prepared for phenotyping generally should not interfere with the position of other white blood cells on the histograms/scattergrams. For additional processing guidelines, see also U.S. Patent Nos. 5,270,208, and 5,262,327, incorporated by reference herein. Of course analogs may be prepared from suitable red cells as the skilled artisan would appreciate.

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### SPECIFIC EXAMPLE 3

The following example describes the preparation of a preferred red blood cell component of the present invention.

In general, concentrated red blood cells are provided, separated from associated supernatant, and concentrated human red blood cell packs are suspended in a solution (e.g. phosphate buffered solution containing PEG (MW = 20,000)) and allowed to settle overnight. The supernatant is then removed and 0.5% NaCl with PEG is added in an equal volume to the packed red blood cells and allowed to set at room temperature for 4-5 hours. The supernatant is again removed and the cells are resuspended in the NaCl solution and stored at 6°C overnight. The packs are further checked for excessive hemolysis and removed from the inventory. The remaining packs are pooled into batches based on the (MCV'S), wherein twelve to fourteen packs are combined to make a batch. The batches are again resuspended in the NaCl solution for about 4-5 hours at room temperature. Of course, other times and temperatures may be employed.

Each batch is resuspended into a phosphate buffered solution containing PEG, EDTA and magnesium gluconate. The cells are allowed to settle, the supernatant is removed and the cells are resuspended in the above solution with lower PEG concentrations for storage up to 90 days at 6°C.

One diluent efficient in stabilizing the red blood cells on the Coulter STKS includes a phosphate buffered solution containing PEG, Na2EDTA, magnesium gluconate and sulfasalazine, wherein the sulfasalazine is added as an antioxidant to prevent hemolysis when the lipoprotein is added to the control composition of the present invention. The final diluent also contains 2% bovine serum albumin to improve the position of the white blood cells. After the cells have been washed into this diluent, trypsin inhibitor from soybeans is added at 0.06% to the total volume of red blood cells to give added stability to the MCV'S.

While the BSA in the diluent improves the white blood cell position on the scattergram, lipoprotein is also preferably used to provide a scattergram that represents whole blood. See U.S. Patent Nos. 5,270,208 and 5,262,327 incorporated by reference. A lipoprotein source consisting of mostly HDL is added at about 100-175 mg/dl to the control composition and a-Tocopherol is further added to the lipoprotein source to reduce peroxides produced by the oxidation of the lipoproteins. If the lipoprotein source is added to the red blood cells without an antioxidant such as sulfasalazine or a-Tocopherol, the peroxides may cause hemolysis of the red blood cells in the present control composition.

#### SPECIFIC EXAMPLE 4

The following example describes the preparation of a nucleated red blood cell component of the present invention.

Turkey red blood cells are washed into a phosphate buffered solution and set to a count of about 1X10<sup>6</sup>/mm<sup>3</sup>. The cells are fixed with a phosphate solution (volume equal to the cell volume) + 0.4% v/v glutaraldehyde, at room temperature for one day and then washed into a phosphate buffer.

The fixed turkey red blood cells are added to the control composition of the present invention to yield a cell count equal to at least 10% of the white blood cell count in order to produce NRBC flags on the Coulter STKS or the Cell-Dyne 4000 manufactured by Abbott Laboratories. Though the present example

contemplates the use of turkey cells, cells from other cell sources may be employed as the skilled artisan will appreciate.

#### SPECIFIC EXAMPLE 5

5       The following example describes the preparation of a simulated platelet component of the present invention.

Goat red blood cells are separated from other constituents of goat whole blood. For instance, cells are washed into PBS three times to remove the plasma and white cells. The concentration is adjusted to  $8 \times 10^6/\text{mm}^3$  and fixed  
10 with a volume of PBS equal to the cells that contain 0.224-0.320% glutaraldehyde, providing the amount of protection needed to allow proper lysis during the shrinkage step. The cells are incubated at 30°C for one hour and centrifuged at 1200 RPM for 15 minutes. The supernatant is removed and the cell volume is adjusted to one-fourth of fixed volume.

15       An ammonium chloride solution is added to the cells to equal the original volume of fixed cells. The ammonium chloride solution creates holes in the membrane to allow hemoglobin to exit to the cells, while the glutaraldehyde protects from total lysis. The cells are monitored for hemoglobin loss based on (MPV) decreases on the H-1. When the MPV is at 10fl on the H-1, the cells are  
20 diluted with a phosphate buffered solution and centrifuged at 1800 RPM for 20 minutes. The supernatant is removed and the cells are washed to remove the free hemoglobin and shrink the membrane around the hemoglobin to produce an MPV of approximately 10fl on impedance instruments, such as the S+IV, manufactured by Beckman Coulter.

25       In a more preferred embodiment, to prevent further loss of hemoglobin and shrinkage of the membrane, the cells are fixed more than once, for instance, with 0.04% glutaraldehyde in a volume of phosphate buffered solution equal to the cell volume at a count of  $1 \times 10^6/\text{mm}^3$ . The cells are left at room temperature overnight and then washed. Though the present example contemplates the use

of goat cells, cells from other cell sources may be employed (such as stabilized human platelets) as the skilled artisan will appreciate.

#### SPECIFIC EXAMPLE 6

5       The following example describes the preparation of a reticulated platelet component of the present invention.

Preferably, reticulated platelets for the control composition of the present invention are prepared by washing goat red blood cells into 0.9% NaCl and concentrated to 70-80% HCT. Equal volumes of concentrated red blood cells  
10 and 4% RNA solution (20 mls of each) adjusted to 300 mosm with KCl are mixed together and dialyzed against 500 mls of a hypotonic solution containing glycerol (osm = 90 - 100) for 90 minutes at 6°C. Dialysis is required to slowly change the osmolarity without damaging the cells. The resulting osmolarity change in the red blood cell solution is from about 300 mosm to about 150 mosm. This process  
15 creates holes in the cell membrane to allow the RNA in the red blood cell solution to enter the red blood cells.

The osmolarity is brought back to isotonicity by dialyzing the red blood cells containing RNA against an isotonic solution. This dialysis is at room temperature for 30 minutes and the final osmolarity of the cells is about 260 mosm. This process reseals the holes that were created by the hypotonic dialysis, thus trapping the RNA inside the cells.  
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Eighty milliliters of the resealing diluent containing 0.1% Nuosept 101 is added to the encapsulated red blood cells and the mixture is heated at 37°C for 3 hours. This heating step helps to lyse the weakened cells from the  
25 encapsulation process and anneals the membranes of the encapsulated red blood cells.

### SPECIFIC EXAMPLE 7

The following example describes a method using the control composition to determine the accuracy and reproducibility of the operation of a multi-parameter automated hematology instrument. By way of example, a multi-parameter automated hematology instrument, such as a Beckman Coulter STKS or Gen-S Systems, the Abbott Cell-Dyn 4000 Hematology System, Bayer ADVIA 120, and the Sysmex XE2100 System, is provided, optionally with a slide preparation module. The claimed control composition is obtained or prepared which includes, by way of example, a treated stabilized human red blood cell component and a reticulocyte component with quality control values in an appropriate range, for example, 1.0%, 2.5%, and 9.0%, respectively. It is refrigerated prior to use. At the beginning of testing, the control composition is allowed to warm to room temperature for about fifteen minutes, mixed manually, and checked for resuspension of contents.

The control composition is prepared and analyzed by the same standard method as test samples which may be tested in batch quantities by the use of a suitable cassette having apertures for receiving test vials. After preparation, the control composition and test samples are analyzed by counting the population number of each subject component type with a multi-parameter automated hematology instrument, which will yield a visual display of the data.

For a Coulter System, the automated test instrument may employ technology known generally as VCS Technology (as marketed by Beckman Coulter). VCS generally analyzes cell samples in view of simultaneous volume conductivity and Scatter measurements. Ordinarily, a starting sample is employed in combination with suitable reagents (which may comprise a component of a kit) and physical agitation for lysing and cell measuring by way of flow cytometry.

Accordingly, the sample may be tested by the Coulter Principle of (DC) Impedance to measure the cell volume in an isotonic suspension.

Conductivity may be employed, for instance, by applying alternating current in the radio frequency range. Energy can penetrate the cell by short circuiting the cell membrane's bipolar lipid layer.

Information about the cells is also possible with light scatter techniques,  
5 such as from the scatter characteristics detected from cells in response to a coherent light source, e.g. a laser beam.

Of course, by no means is the mode of sample testing limited to the above. As mentioned other principles may be used.

The respective population counts obtained from the analysis are  
10 compared either to known reference value for each component type in the control composition, or by comparison of the population counts for each component types in the test sample with the corresponding values of components in the control composition. Data relating to the measurement of components in control composition and test samples is collected, monitored,  
15 stored, compared and analyzed by electronic means, such as a computer programmed with appropriate software and containing appropriate data file structure.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

All patents and other publications cited herein are expressly incorporated  
25 by reference.